#13/105
11/5/02

PATENT
Attorney Docket 044574-5061

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application: **John Carlson *et al.***	)	
	)	
Application No. **09/491,577**	)	Group Art Unit: **1646**
	)	
Filed: **January 25, 2000**	)	Examiner: **Joseph Murphy**
	)	
For: **Drosophila Odorant Receptors (Amended)**	)	

DECLARATION UNDER 37 C.F.R. 1.132

I, John R. Carlson, do hereby make the following declaration:

1. I have served as a Professor in the Department of Molecular, Cellular and Developmental Biology at Yale University since 1986. I have more than eighteen years of experience in the field of *Drosophila* research. I have published over thirty scientific articles relating to *Drosophila* research in peer-reviewed journal publications and texts. I have served on numerous advisory committees for the National Institutes of Health including study sections, site visit committees, and special emphasis panels. I earned a A.B. in biochemical sciences from Harvard University, and a Ph.D. in Biochemistry from Stanford University. I also completed my post-doctoral training at Stanford University. A biographical sketch of my experience and education is attached for the Examiner's review.

2. I have reviewed the Office Action dated June 29, 2001, and in particular the Examiner's questions concerning the lack of utility purportedly because the polypeptides encoded by the nucleic acids lack biological significance in the absence of a natural substrate. I hereby state that I instructed personnel under my direction and control to conduct two series of experiments on Or22a, also known as DOR22a (SEQ ID NO: 31) and on the immediately adjacent and closely related Or22b, also known as DOR22b (SEQ ID NO: 1) genes. Both lines of experimentation indicate that the Or22a/b receptor binds to ethyl butyrate. These experimental studies were conducted as set forth below and representative data from these studies is included in the attached figures.

3. Antibodies cross-reactive to Or22a and Or22b proteins label dendrites of *Drosophila* olfactory receptor neurons (Carlson Declaration Figure A). Or22a and Or22b are highly similar in sequence, and an antibody was raised against a short region expected to be identical between the two receptor proteins. Carlson Declaration Figure A is a display of a cross-section through an olfactory sensillum on the antenna labeled with the anti-Or22a/b antibody.

4. Or22a/b expression has the same distribution pattern as ab3 sensilla (Carlson Declaration Figure B). The left panel shows immunofluorescence labeling of the antenna with the anti-Or22a/b antibody. A small subset of sensilla in the dorso-medial portion (upper right corner) of the antenna is labeled. The morphology of these sensilla indicate that they are of a morphological class called large basiconic sensilla. The distribution of the labeled sensilla corresponds closely to that of one particular functional type of large sensilla basiconica, the ab3 sensilla, shown in the right panel (de Bruyne *et al.* (2001) Neuron 30, 537-552).

5. The promoter region of Or22a was used to drive expression of GFP (green fluorescent protein) via the UAS-GAL4 system (Carlson Declaration Figure C). The promoter/enhancer of the Or22a gene was covalently joined to the gene encoding the yeast transcription factor GAL4, which binds to UAS (upstream activation sequences). Five UAS sequences were inserted upstream of a reporter gene, in this case GFP. Expression was observed in the same olfactory sensilla that were labeled with antibody raised against Or22a (Carlson Declaration Figure D). The top left panel shows immunofluorescence with the anti-Or22a/b antibodies. The top right panel shows GFP labeling driven by the Or22a promoter. The two patterns are identical, as confirmed by the merged image below and demonstrates that the GFP in fact marks the Or22a/b-immunoreactive sensilla.

6. Sensilla labeled with Or22a-GFP are ab3. Physiological recordings are shown for each of the three types of large basiconic sensilla, ab1, ab2, and ab3, on the antenna of control flies, and from the GFP-labeled sensilla of Or22a-GAL4/UAS-GFP flies (Carlson

Declaration Figure E). The ab1 sensillum contains four classes of neurons; ab2 and ab3 contain two classes of neurons each. Each bar indicates the increase, in action potentials/second, for each class of neuron within a sensillum, relative to the spontaneous firing frequency. The recordings from Or22a-GFP sensilla indicate that these labeled sensilla contain two neurons, consistent with either ab2 or ab3; their odor-sensitivities correspond well to those of ab3. Please note that the odors used in this experiment are selected from a panel of forty-seven tested in deBruyne *et al.* (2001) Neuron 30, 537-552, and include many of the odors which stimulate the ab3 sensilla; most of the odors tested in deBruyne *et al.* did not stimulate the neurons of the ab3 sensillum.

7. Or22a-GAL4/UAS-*rpr* lack ab3A cells (Carlson Declaration Figure F) indicating that Or22a/b is expressed in ab3A neurons, which are known to be sensitive to ethyl butryate. ab3 sensilla contain two neurons, the ab3A neuron and the ab3B neuron. To determine in which neuron Or22a/b is expressed, the Or22a promoter was used to drive synthesis of the reaper gene via GAL4 (note: this was done in a manner analogous to the GFP constructs). When reaper gene expression was driven by the Or22a promoter, physiological recordings indicate that the ab3A neuron activity was absent. The traces show electrical activity of neurons in the ab3 sensillum in response to a 0.5 second application of ethyl butyrate (indicated by the horizontal bar above the traces). The upper trace from a control fly shows two classes of spikes, those of large amplitude, representing the activity of the ab3A neuron, and those of small amplitude, representing the activity of the ab3B neuron. In the lower trace the spikes of large amplitude are missing, indicating the loss of the ab3A neuron. These experiments further indicate that Or22a/b is expressed in ab3A.

8. Deletion mutants lacking Or22a/b display severely reduced response to ethyl butyrate (Carlson Declaration Figure G). A deletion mutant lacking the Or22a and Or22b genes, designated Delta(halo) displayed a severely reduced response to ethyl butyrate, as shown in the top trace and in the rightmost panel. The trace shows electrical activity of neurons in the ab3 sensillum in response to a 0.5 second application of ethyl butyrate (the

application of stimulus is indicated by the horizontal bar below the trace). The trace shows two classes of spikes, those of large amplitude, representing the activity of the ab3A neuron, and those of small amplitude, representing the activity of the ab3B neuron. The frequency of firing of the A neuron is low after stimulation with ethyl butyrate (compared with the control shown in Carlson Declaration Figure F). Responses to a panel of odors are shown in the three panels below, for a control strain, the Or22a-GAL4/UAS-*rpr* strain, and the deletion strain (n=10 to 12 sensilla; green bars indicate ab3A neurons; blue bars indicate ab3B neurons). These results demonstrate that Or22a/b is required for response of ab3A to ethyl butyrate and the other odors to which it responds. The odors used in this experiment were again selected from a panel of forty-seven tested in de Bruyne *et al.* and include many of the odors which were found to stimulate the ab3 sensilla. Most of the forty-seven odors tested did not stimulate the neurons of the ab3 sensillum. Moreover, we have found evidence that the wild-type response to ethyl butyrate is rescued by supplying a transgenic wild-type copy of Or22a, consistent with a function for Or22a in binding ethyl butyrate.

9. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

12/21/01
Date

John R. Carlson
John R. Carlson, Ph.D.

Anti-Or22a/b Antibodies Label Dendrites of Olfactory Receptor Neurons

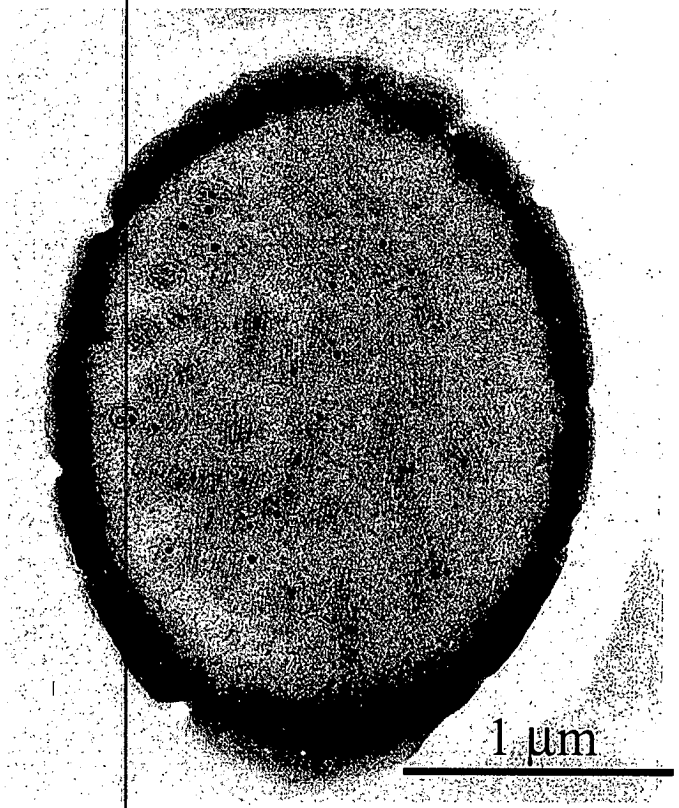
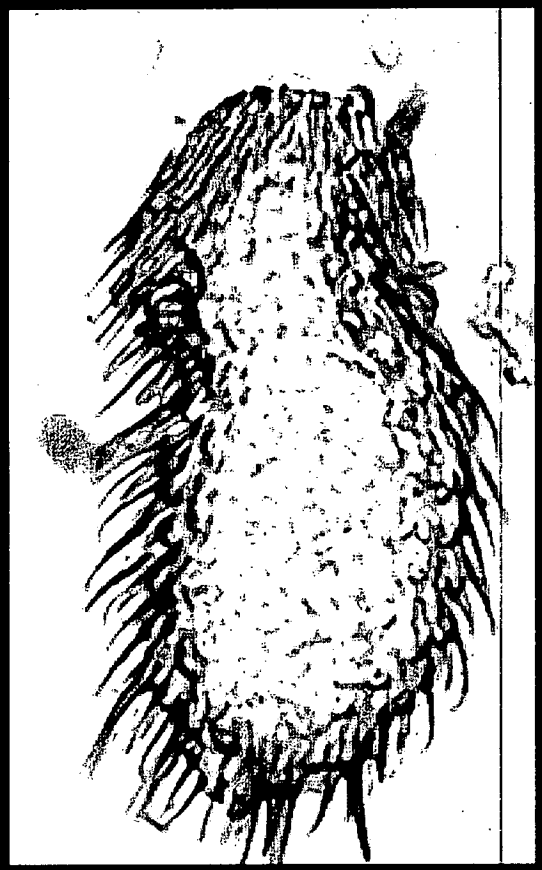


Figure A

Or 22a/b shows the same distribution as ab3 sensilla



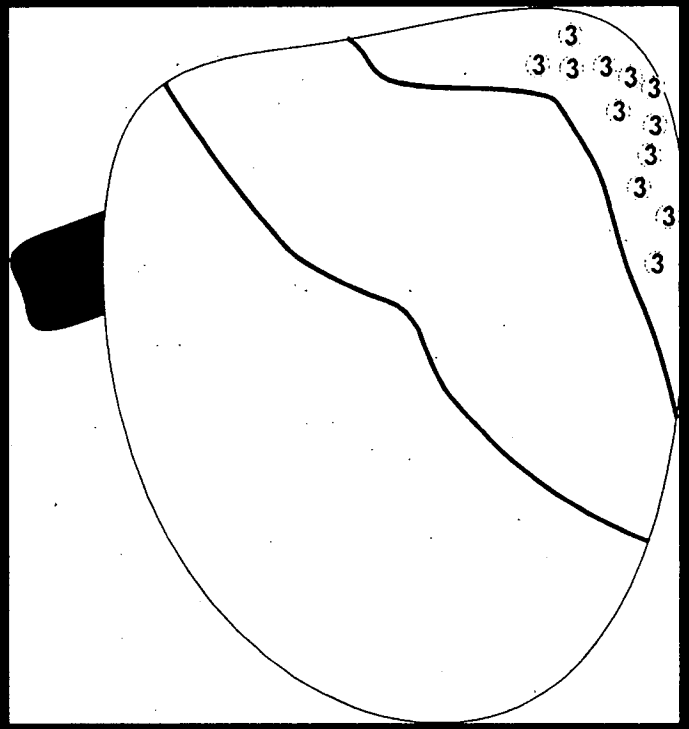


Figure B

GAL4/UAS system

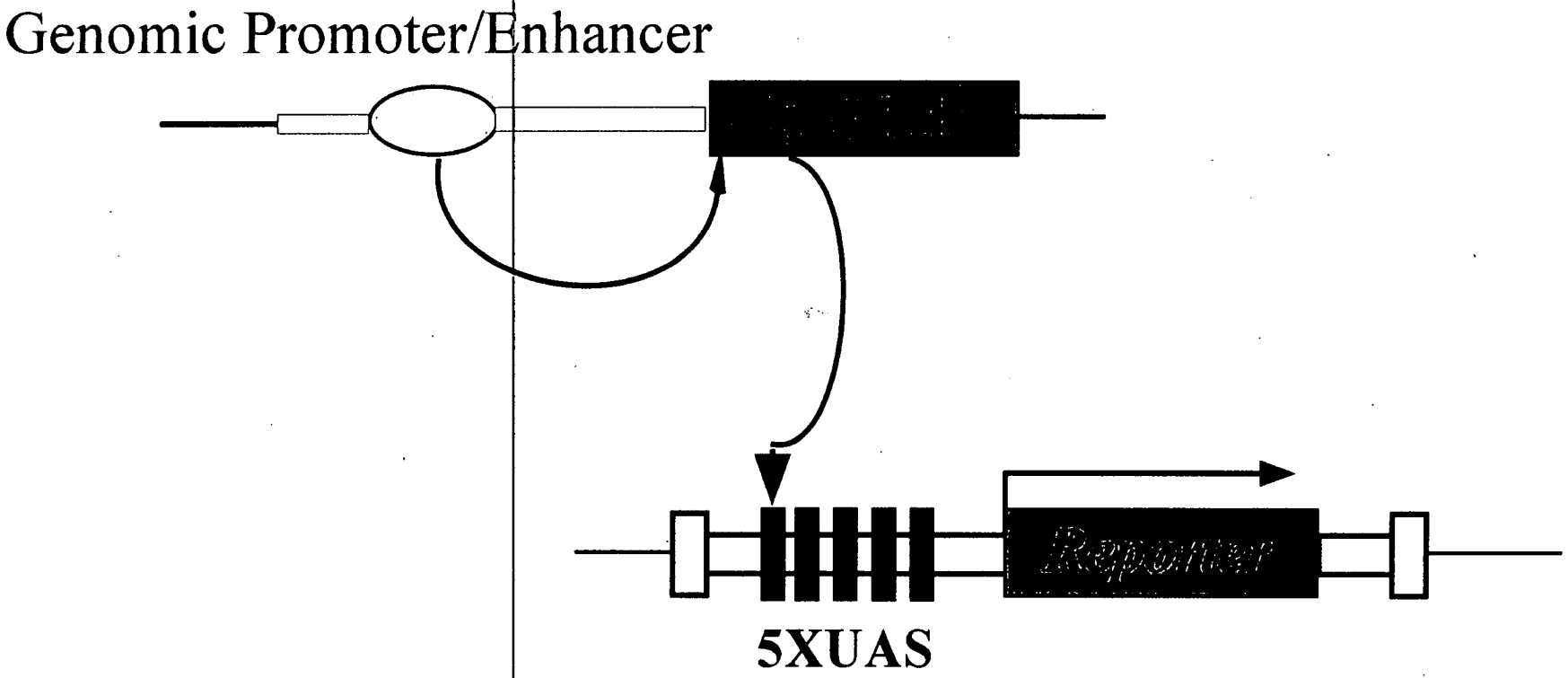


Figure C

Or22a-Gal4 is expressed in the sensilla recognized by anti-Or22a/b antibodies

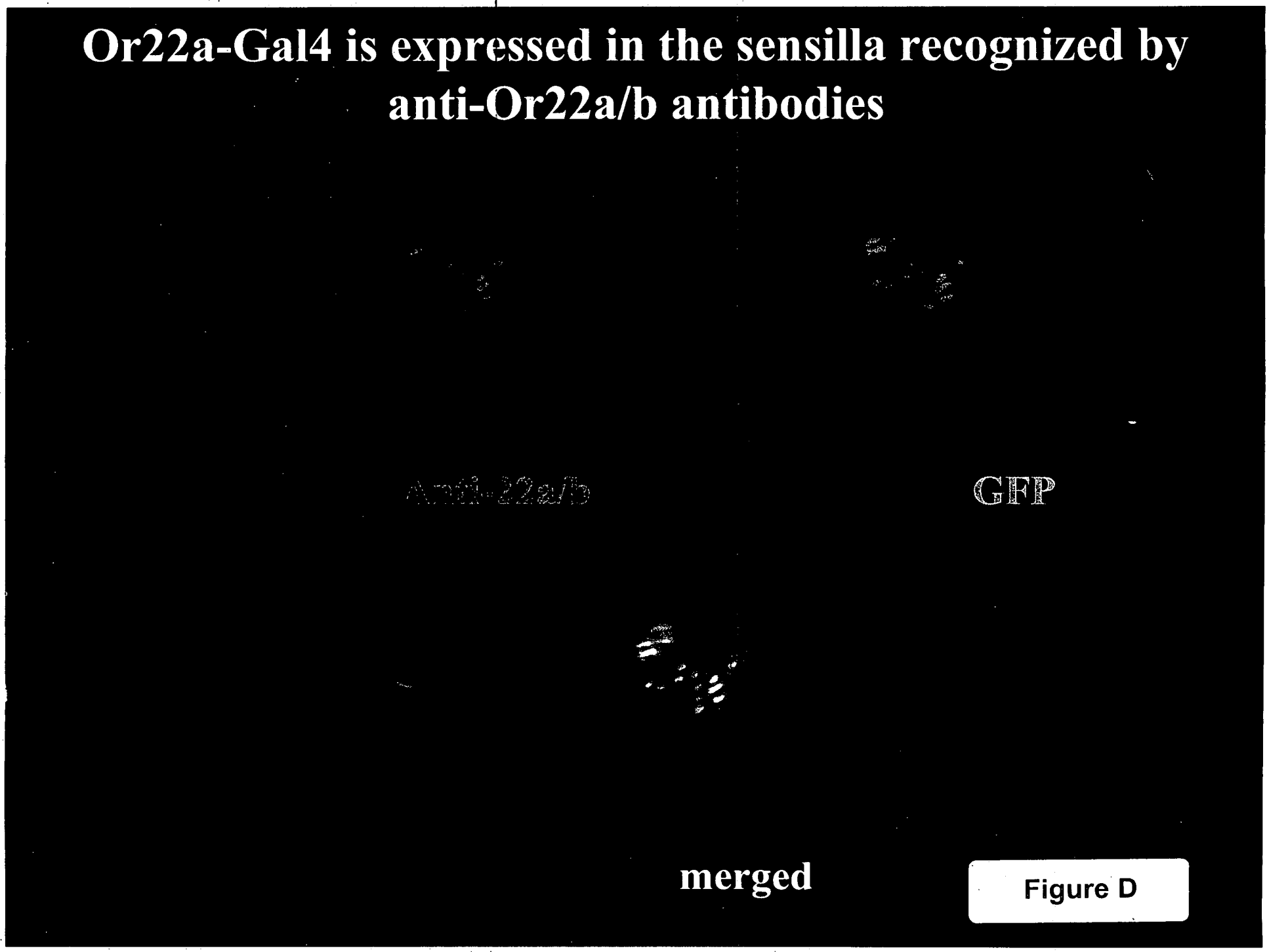


Figure D

The sensilla labeled with Or22a-GFP are ab3

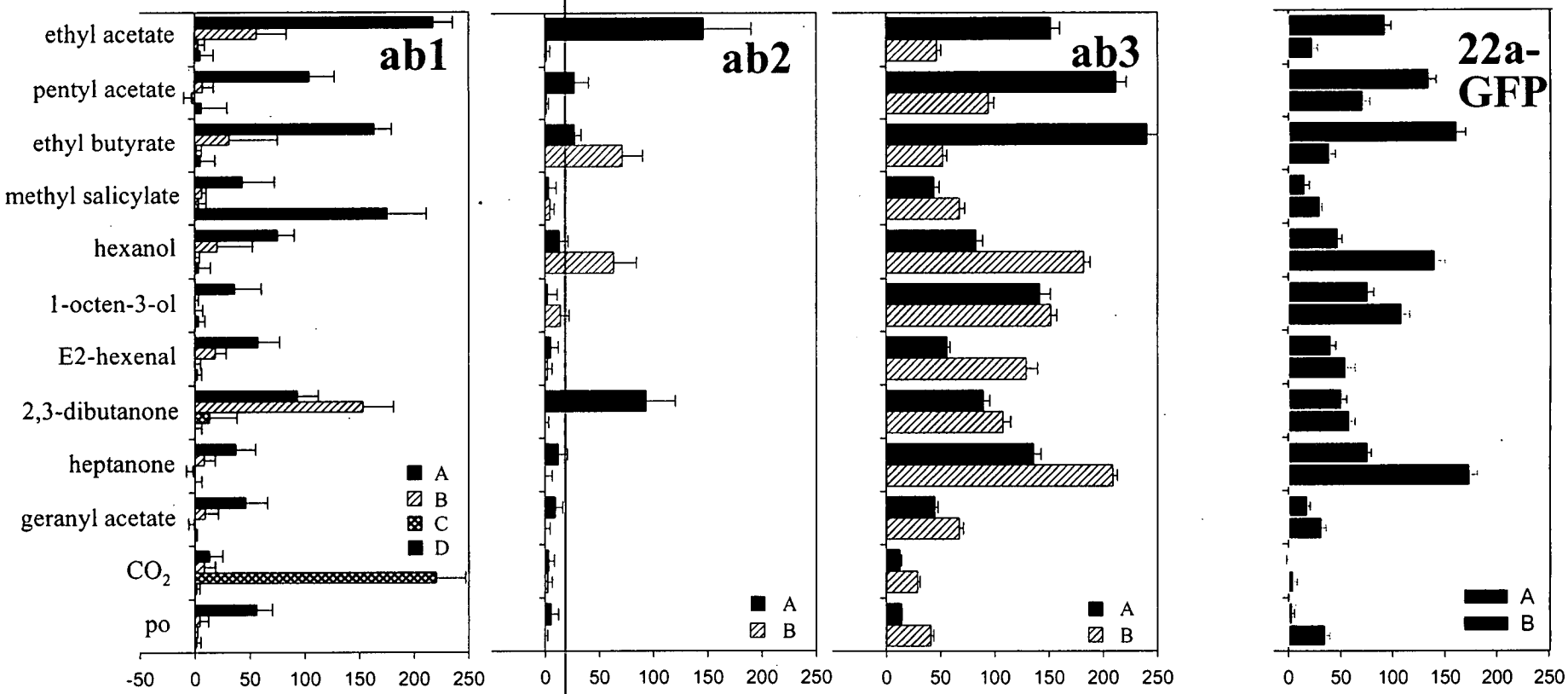


Figure E

Or22a-GAL4/UAS-*rpr* Flies Lack ab3A Cells: Or 22a/b is Expressed in ab3A Neurons

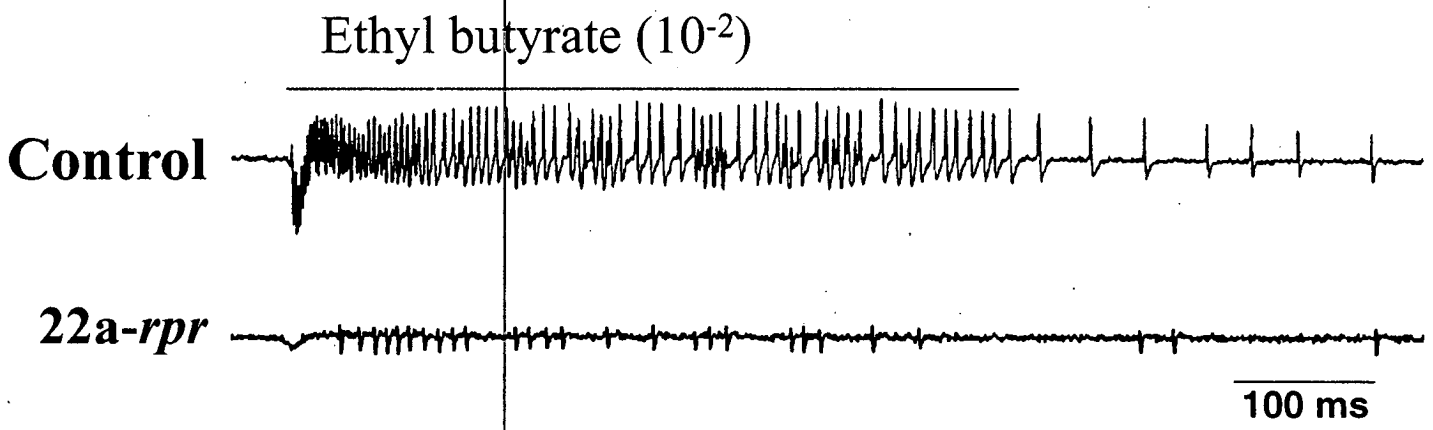


Figure F

Deletion mutant lacking Or22a/b

Ethyl butyrate

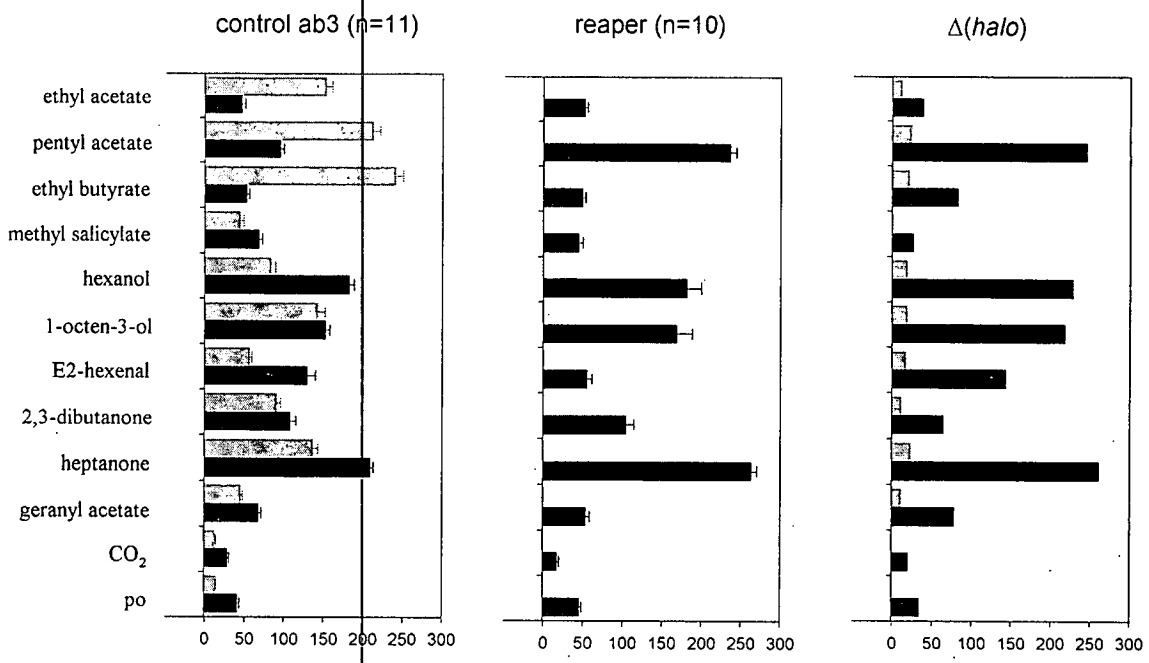


Figure G

Gray Bars = Green
Black Bars = Blue

John R. Carlson, Ph.D.
Professor of Molecular, Cellular, and Developmental Biology

Education

Harvard College, Cambridge, MA	A.B.	1977	Biochemical Sciences
Stanford University, Stanford, CA	Ph.D.	1982	Biochemistry
Stanford University, Stanford, CA	Postdoc		Molecular Biology

Professional Experience

6/76 - 6/77	Undergraduate research with Dr. Walter Gilbert, Harvard University
1/78 - 9/82	Graduate research with Dr. David Hogness, Dept. of Biochemistry, Stanford University
9/82 - 2/86	Postdoctoral research with Dr. Irving Weissman, Stanford University
2/86 - present	Assistant, Associate and Full Professor, Yale University

Honors

National Merit Scholarship
John Harvard Scholarship
Phi Beta Kappa
National Science Foundation Graduate Fellowship
Helen Hay Whitney Foundation Postdoctoral Fellowship
Alfred P. Sloan Research Fellowship
McKnight Scholars Award
Yale College Dylan Hixon Prize for Excellence in Teaching in the Natural Sciences, 1998
McKnight Investigator Award, 2000
Lecture, The NIH Director's Lecture Series 2000

Federal Government Public Advisory Committees

NIH Special Emphasis Panels, NIDCD, 1994, 1997, 1999
NIH Study Section, Neurology C, Ad Hoc Member, 1993, 1998
NIH Neurology C Special Emphasis Panel, 1997
NIH Site Visit Committees (Program Project Grant Reviews) 1989, 1994, 1996
NIH Initial Review Group, NICHD, MCH subcommittee, Temporary Member 1996
NIH Study Section, Visual Sciences C, Ad Hoc Member, 1993
NIH Study Section, Biology 2, Ad Hoc Member, 1992

Selected Publications

McKenna, M., Monte, P., Helfand, S., Woodard, C., and Carlson, J., (1989) A Novel Chemosensory Response in *Drosophila* and the Isolation of *acj* Mutations which Affect It, *Proc. Natl. Acad. Sci. USA,* 86, 8118-8122.

Helfand, S. and J. Carlson, (1989) Isolation and Characterization of an Olfactory Mutant in *Drosophila* with a Chemically-Specific Defect, *Proc. Natl. Acad. Sci. USA*, 86, 2908-2912.

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Clyne, P., Warr, C., Freeman, M., Lessing, D., Kim, J., and Carlson, J., (1999) A Novel Family of Divergent Seven Transmembrane Proteins: Candidate Odorant Receptors in Drosophila, *Neuron*, 22, 339-347.

de Bruyne, M., Clyne, P., and Carlson, J., (1999) Odor Coding in a Model Olfactory Organ: The Drosophila Maxillary Palp, *J. Neuroscience*, 19, 4520-4532.

Störtkuhl, K., Hovemann, B., and J. Carlson, (1999) Olfactory Adaptation Depends on the Trp Ca^{2+} Channel in Drosophila, *J. Neuroscience*, 19, 4839-4846.

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Carlson, J. (2001) Viewing odors in the mushroom body of the fly, *Trends in Neurosci.*, 24, 497-498.

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